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(71)(72) Applicants and Inventors: GOULD, Hannah, Jane [GB/GB]; The Randall Institute, Kings College London, 26–29 Drury Lane, London WC2B 5RL (GB). SUTTON, Brian, John [GB/GB]; The Randall Institute, Kings College London, 26–29 Drury Lane, London WC2B 5RL (GB).

(74) Agents: O'BRIEN, Caroline, J. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).

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(54) Title: SOLUBLE VARIANTS OF THE IGE RECEPTOR FC-EPSILON-RI-ALPHA WITH INCREASED AFFINITY

(57) Abstract

A soluble polypeptide has an IgE–binding functionality corresponding to that of the α–chain of the mast–cell Fc–ϵ–R1 IgE receptor protein, and enhanced binding to the Fc portion of IgE, and comprises substantially the sequence and conformation of at least one and preferably both of the two C2–type immunoglobulin–like domains of the α–chain of the Fc–ϵ–R1 IgE receptor protein, substantially lacks a sequence corresponding to the transmembrane segment of said receptor protein, and has a mutation relative to a natural sequence of said receptor protein, especially for example (a) lys117 to large hydrophobic residue such as phe, tyr or trp; (b) glu132 to a large hydrophobic residue such as tyr; (c) lys154 to a large hydrophobic residue such as phe, leu, or tyr; (d) gly124 to a positively charged residue such as lysine or arginine; (e) lys128 to a negatively charged residue such as glutamate; (f) gln157 to a negatively charged residue such as glutamate or aspartate; (g) leu158 to a negatively charged residue such as glutamate or aspartate; (h) asp159 to a positively charged residue such as lysine or arginine. Also provided are corresponding polynucleotides and host cells and methods and pharmaceutical compositions for use in reducing excessive IgE action, e.g. allergic reaction.

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SOLUBLE VARIANTS OF THE IGE RECEPTOR FC-EPSILON-RI-ALPHA WITH INCREASED AFFINITY

Field of the invention:

This invention concerns new soluble polypeptides having IgE-binding functionality corresponding to that of mast cell IgE-receptors, particularly to the Fc-epsilon-R1 receptor alpha-chain (also referred to as FceRIa, and its soluble fragment (e.g. extracellular domain) referred to as sFcϵRIα); to materials and methods for their production; particularly by means of expression by recombinant DNA technique; to their derivatives and to pharmaceutical compositions containing them and their derivatives; and to the uses of such materials; for example in the modulation of atopic and other IgE-related immunological responses.

Background to the invention and prior art:

IgE is one of several classes of immunoglobulin, and is known to play an active part in a number of immune responses, particularly in atopic responses ('allergies').

It is known that mast cells, also involved in immune responses, particularly for example of the atopic type, express on their surfaces a receptor protein for IgE. It is known that human, monkey, rat, mouse and other mammalian species have high-affinity IgE receptors of which the alpha-chain binds IgE and in the case of some species is of already-known sequence (A Shimizu et al, Proc Nat Acad Sci US 85 (1988) pp 1907-1911; JM McDonnell et al, Nature Structural Biology 3(5) (May 1996) pp 419-426).

Interaction between IgE and its high-affinity receptor, FcϵRI, on mast cells and basophils generates receptors for allergen which can then trigger an allergic response by activation of these cells at sites of allergen challenge (Metzger, 1992; Ravetch and Kinet, 1991; Sutton and Gould, 1993). The products of cell activation initiate an inflammatory cascade in which other cells bearing the receptor (eosinophils. monocytes and platelets) participate. Allergens also activate Langerhans cells bearing FcϵRI and IgE, and these cells migrate to the lymph nodes to re-establish T cell memory of the allergen, thus perpetuating hypersensitivity (Bieber et al, 1992).

The receptor FcϵRI α-chain contains the binding site for IgE (Hakimi et

al, 1990; Blank et al, 1991). The extracellular sequence of FcεRI is predicted to consist of two immunoglobulin-like domains of the C2 type, as found in CD2 and CD4 (McDonnell et al, 1996). Robertson (1993) has recently shown that a truncated receptor fragment containing only the second, membrane-proximal domain (α(2)) binds to IgE, but with much lower affinity than a soluble fragment of the receptor containing both domains α(I) and α(2). The α(2) domain is predicted to consist of two β-sheets comprising the ABE strands on one face of the domain, and the C'CFG strands on the other. The ABE face contains the three putative glycosylation sites in α(2). Although the receptor is heterogeneously glycosylated (as deduced from the spread of molecular weights on SDS PAGE), the molecular mass determined by sedimentation equilibrium indicates that to a good approximation all seven putative glycosylation sites are occupied (Keown et al, 1995). Carbohydrate chains are therefore likely to mask the ABE face of α(2), implicating the C'CFG face in binding IgE. Involvement of the C'CFG face is also consistent with mutagenesis of the homologous subunit of the IgG receptors, FcγRII and FcγRIII (Hogarth et al, 1992; Hulett et al, 1994), and a preliminary report of mutagensis in the α(2) domain of FcεRI (Danho et al, 1995). In addition, a group including the present inventors recently generated an 11-amino acid constrained peptide with the sequence of the C'C strands and intervening loop of α(2), which competes very effectively for IgE binding to the receptor and prevents the sensitization of masts cells by IgE (McDonnell et al, 1996).

Also known is a recombinant soluble fragment form of the human Fc-epsilon-R1 IgE receptor protein alpha chain, and its effect in downregulating IgE synthesis (Y Yanagihara et al, J Clin Invest 94 (1994) 2162-2165).

Furthermore, International patent application WO 89/05352 (Harvard College and NIH: P Benfey and P Leder) discloses cDNA encoding an IgE receptor alpha-subunit or its IgE binding fragment and proposes use of the subunit to treat allergies, or to produce antibodies for use in in-vitro diagnostics. The alpha-subunit (or soluble fragments) are also proposed for therapeutic use to treat allergies or to design non-peptide drugs which behave therapeutically like the peptides.

US 5,639,660 (Hoffmann-la Roche and DHHS: JP Kinet et JP Kochan) describes and claims cDNA encoding a polypeptide comprising the alpha

subunit of the human high affinity receptor for IgE (FceRI), and proposes it for use to produce human FceRI alpha polypeptides for therapy or to prevent allergic responses, in drug screening assays or for monitoring IgE levels in patients.

International patent application WO 96/08512 (Austin Research Institute: P M Hogarth et al) relates to polypeptides with Fc binding ability, and proposes novel polypeptides, with Fc binding activity altered (compared to a native Fc receptor) by addition, deletion and/or substitution of one or more amino acids compared to the native receptor. The altered Fc receptor-like polypeptides are proposed as having enhanced or decreased Fc binding ability or increased serum half life, and for uses including detecting Ig, removing Ig from samples, or treating diseases where an excess of Ig is implicated as a causative agent.

These prior specifications indicate inter alia that methods of assessing the binding activity of FceRI for its binding partner Fc of IgE are known.

It has also been disclosed that synthetic cyclic peptides with 13 aminoacid residues can model a binding region of the human Fc-epsilon-R1 IgE receptor protein, and that these peptides can inhibit IgE-mediated mast cell degranulation, represented as an in-vitro model of an allergic response (JM McDonnell et al, Nature Structural Biology 3(5) (May 1996) pp 419-426). However, the reported affinities of these disclosed peptides for IgE were relatively low, substantially lower than the affinity of the natural receptor. In this connection, a test of the ability of the peptides to inhibit passive senstization of rat basophilic leukaemia (RBL) cells with IgE, with subsequent attenuation of allergen-induced deranulation measured by dose-dependent inhibition of hexosaminidase release from the RBL cells, gave results that the 50% inhibitory concentration of the peptides was in the range 30-100 micromolar, while the corresponding 50% inhibitory concentration of soluble Fc-epsilon-R1 alpha-chain was about 10 nanomolar (McDonnell et al, 1996, cited above), i.e. a ratio of effective concentrations in the range about 3000-10,000, with the peptides being less effective than the natural receptor.

The present inventors consider that there remains a need for further modified binding materials for usefully binding IgE and related substances. An aim of the present invention is to provide competitive inhibitors of the

interaction between IgE and FcεRI, for use prophylactically in the treatment of allergy. One of the approaches taken by the present inventors is to locate the complementary binding sites in IgE and the receptor.

Summary and description of the invention:

According to an aspect of the invention, a IgE-receptor alpha-subunit can be prepared as a soluble fragment with an aminoacid sequence modified to increase its affinity for the IgE constant-region and to improve binding relative to the natural receptor.

The present invention therefore in one aspect provides soluble polypeptides having an IgE-binding functionality corresponding to that of the alpha-chain of the mast-cell Fc-epsilon-R1 IgE receptor protein, said polypeptides (a) comprising sequences of substantially the sequence and conformation of at least one and preferably both of the two C2-type immunoglobulin-like domains of the alpha-chain of the Fc-epsilon-R1 IgE receptor protein, and (b) substantially lacking a sequence corresponding to the transmembrane segment of said receptor protein, and (c) also having a mutation relative to a natural sequence of said receptor protein, which mutation enhances the binding of the polypeptide to the Fc portion of said IgE.

The expression 'C2-type' (as found in CD2 and CD4) in relation to such domains is explained in McDonnell et al (1996) and references cited therein, incorporated herein by reference.

A useful mutation can for example be introduced in one or more of the aminoacid sequence regions contributing to the alpha-2 (α(2)) domain, for example to the C'CFG face, of the alpha-chain of the Fc-epsilon-R1 IgE receptor protein. Among useful mutations are those that either increase the overall affinity of the receptor for its ligand or leave the overall affinity substantially unchanged, which can preferably be used in combination with another mutation, e.g. one that increases the affinity.

The residues in the C'CFG region of the FceRIa or sFcεRIα subunit are formed (as is known from guidance obtainable in the application from which priority is claimed, and/or references cited therein, and correspondingly in a subsequently-published paper of which the present inventors are co-authors, JPD Cook et al, Biochemistry 1997 36:15579-15588, which is hereby

incorporated in its entirety by reference) from those of the FceRI or sFcϵRIα aminoacid chain portions containing the following residues:-

in the C strand: asp114, val115, tyr116, lys117, val118, ile119, tyr120, tyr121, lys122, and asp123:

in the C' strand: gly124, glu125, ala126, leu127, lys128, tyr129, trp130, tyr131, and glu132:

and in the F and G strands, including the F-G loop: glu144, asp145, ser146, gly147, thr148, tyr149, tyr150, cys151, thr152, gly153, lys154, val155, trp156, gln157, leu158, asp159, tyr160, glu161, ser162, glu163, pro164, leu165, asn166, ile167, thr168, val169 and ile170.

According to an aspect of the invention, selection of aminoacids to be mutated in the manner described herein can be made on the basis of the enumerated aminoacids, subject to the exceptions of buried residues tyr116, val118, tyr120, ser146, tyr149, cys151, gly153, val155, ile167 and val169. (These exceptions are considered to constitute non-exposed residues e.g. on the basis of the homology available in 'Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor', J M McDonnell et al, Nature Structural Biology 1996, 3(5):419-426, along with BJ Sutton and HJ Gould, Nature 1993, 366:421-428.) Thus the aminoacid residues of the C'CFG face are those listed, subject to the non-exposed exceptions as given above.

The invention provides in one aspect a soluble polypeptide having an IgE-binding functionality corresponding to that of the alpha-chain of the mast-cell Fc-epsilon-R1 IgE receptor protein, and of enhanced binding to the Fc portion of said IgE, said polypeptide (a) comprising a sequence of substantially the sequence and conformation of at least one and preferably both of the two C2-type immunoglobulin-like domains of the alpha-chain of the Fc-epsilon-R1 IgE receptor protein, and (b) substantially lacking a sequence corresponding to the transmembrane segment of said receptor protein, and (c) also having a mutation, relative to a natural sequence of said receptor protein, in one or more of the aminoacid residues of the C'CFG region of the FceRI subunit: (in the C strand): asp114, val115, lys117, ile119, tyr121, lys122, and asp123: (in the C' strand): gly124, glu125, ala126, leu127, lys128, tyr129, tyr131, and glu132: (in the F and G strands, including the F-G loop): glu144, asp145,

gly147, thr148, tyr150, thr152, lys154, gln157, leu158, asp159, ser162, glu163, pro164, leu165, asn166, thr168 and ile170.

The invention relates in certain examples to mutation of residues other than individual residues proposed to be mutated in WO 96/08512 (Austin Research Institute: P M Hogarth et al), e.g. other than mutation of trp156, and provides in certain examples mutation of residues of the given list of residues of the C'CFG face (i.e. after exception of non-exposed residues) other than trp130, trp156, tyr160, or glu161.

According to the invention there are also provided mutants of FceRI and sFcϵRIα which are mutated in respect of aminoacid residues of the C'CFG face (i.e. those of the given list after exception of non-exposed residues) and have affinities of at least 2x, e.g. at least 3x or 4x the affinity of the unmutated FceRI or its soluble fragments sFcϵRIα (e.g. extracellular domain).

On the basis of molecular modelling of the complex between sFcϵRIα and IgE-Fc, the inventors provide a further aspect of the present invention, by which it is preferred to mutate residues ('contact residues') of the C'CFG face that are particularly surface-accessible in relation to the binding of the IgE-Fc ligand and form a surface complementary to the IgE-Fc ligand.

Thus, in this preferred aspect of the present invention, the residues to be mutated are those from the C'CFG region that form particularly exposed residues of the C'CFG face, i.e. for example lys117, ile119, tyr121, gly124, glu125, ala126, leu127, lys128, tyr129, tyr131, glu132, lys154, trp156, gln157, leu158, and asp159.

It is believed to be particularly preferable not to mutate trp156 if binding affinity is to be maintained or increased, since the inventors' molecular-modelling studies indicate that the size and hydrophobicity of trp156 is important for Fc-IgE binding by FceRI or sFcϵRIα, and no other aminoacid residue of suitable size and properties is available as a substitute.

The present invention thus provides mutants of FceRI or sFcϵRIα in respect of these residues (e.g. other than mutants in which trp156 is mutated).

Preferred mutations of aminoacids forming contact residues in the C'CFG face are of the following kinds:

(a) A first kind comprises mutations to enhance hydrophobic contact

surface between the receptor and its ligand: e.g. a mutation of lys117 to a large hydrophobic residue such as phe, tyr or trp; or a mutation of glu132 to a large hydrophobic residue such as tyr; or a mutation of lys154 to a large hydrophobic residue such as phe, leu, or tyr.

(b) A second kind comprises mutations to enhance electrostatic complementarity between the receptor and its ligand: e.g. a mutation of gly124 to a positively charged residue such as lysine or arginine (because Fc has a complementary negative charge in the region of this residue); or a mutation of lys128 to a negatively charged residue such as glutamate; or a mutation of gln157 to a negatively charged residue such as glutamate or aspartate; or a mutation of leu158 to a negatively charged residue such as glutamate or aspartate (in the cases of the last three positions, because Fc has a complementary positive charge in the region of the residue concerned and the sidechain sizes substantially match). Also proposed is a mutation of asp159 to a positively charged residue such as lysine or arginine.

Further examples of mutations within the scope of the invention are: mutation of any of the following hydrophobic residues to a hydrophobic residue of similar or larger size: ile119 to leu or phe; tyr121 to phe or trp; ala126 to val, leu or ile; leu127 to ile or tyr; or tyr129 to phe or trp; and mutation of any of the following residues to form or enhance a salt bridge interaction: glu125 to asp; tyr131 to asp or glu; or asp159 to lys or arg.

The invention in particular examples relates to mutant analogues of soluble fragments of IgE receptor protein subunits of the human, rat, mouse and other non-human mammalian species. Many other animal species including monkeys have analogous receptors and the invention extends to mutants of soluble fragments of the homologous receptors in other species, for example besides human the rat mouse and monkey.

In the case of Fc-epsilon-R1 alpha-chain receptors of species for which cDNA and expression vectors are not available, PCR technique can readily be used without undue difficulty to isolate cDNA on the basis of consensus oligonucleotides in the nearest available species, since it has been observed that the homologies amongst the known members are close.

Sometimes there is a cross-species functionality, i.e. the Fc-epsilon-R1 alpha-chain receptor of one species can in several cases bind the IgE Fc region

of another species, though sometimes with lower affinity, e.g. about tenfold affinity.

Thus, more generally, polypeptides of the invention can be used for inhibiting IgE binding to Fc-epsilon-R1 on cells. The polypeptides find further uses against conditions, such as GVH disease and transplant rejection, which correlate with IgE production as is already known. They can be used for therapeutically or prophylactically treating an individual suspected of suffering from, or susceptible to an immediate or late phase allergic response or other IgE-related disease, e.g. graft versus host disease (GVHD), allergic rhinitis, food allergies, atopic dermatitis or allergic asthma.

Polypeptides of the invention can further have binding effects and uses anologous to those disclosed for soluble receptor-derived sub-sequence peptides in for example specification WO 96/01643, King College and Thomas Jefferson Univ: AJ Beavil et al) and also unmutated soluble alpha chain fragments which have corresponding effects.

Polypeptides of the present invention can also block IgE binding to the other, low affinity, receptor, Fc-epsilon-R2, also known as CD23, in addition to their blocking effect on IgE binding to the high affinity receptor Fc-epsilon-R1.

The low affinity receptor has a role in relation to IgE allergen complexes involved in antigen presenting cells, and accordingly some additional uses can arise wherever it is desired to downregulate IgE expression or the development of IgE expression.

A particularly useful polypeptide according to an example of the invention is one that comprises the sequence of both of the two C2-type immunoglobulin-like domains of the alpha-chain (α(1) and α(2)) of the human Fc-epsilon-R1 IgE receptor protein, except for a substitution mutation involving replacement of an aspartate residue by a lysine residue, and denoted as asp159lys or D159K (using standard single-letter aminoacid abbreviations and using the protein numbering sequence of the alpha-chain of the human mast-cell Fc-epsilon-R1 IgE receptor protein as given by the prior art).

A polypeptide according to an example of the invention can have a rate constant kd2 for the dissociation of the high-affinity complex between IgE and the polypeptide having the binding functionality of the alpha-chain of the

human Fc-epsilon-R1 IgE receptor protein, which rate constant is substantially less than the corresponding rate constant of the polypeptide of natural sequence: this dissociation rate constant can be for example less than about half that of the polypeptide of natural sequence, e.g. about 0.2 times that of the polypeptide of natural sequence, as in the case for example of the asp159lys (D159K) mutant.

In this connection, it has been observed that the binding reaction between such polypeptides and IgE comprises kinetically biphasic association and dissociation processes (which can as is usual with association-dissociation phenomena lead to a dynamic equilibrium), and which have been observed to involve, during association, the formation of a relatively lower-affinity complex and subsequently the change of this into a relatively higher-affinity complex, and the converse processes in connection with the corresponding dissociation. The rate constant referred to above as kd2 is that which characterises the dissociation of the higher-affinity complex of IgE and the polypeptide having the binding functionality of the alpha-chain of the human Fc-epsilon-R1 IgE receptor protein, to form the lower-affinity complex. The corresponding rate constant in the case of the receptor protein of natural sequence has been measured at about 3.2x10^-5 per second. Thus the kd2 dissociation rate constant for the above-mentioned asp159lys (D159K) mutant polypeptide example of the invention is about 6.4x10^-6 per second.

Correspondingly, polypeptides according to examples of the invention can usefully have higher affinity for IgE than the alpha-chain of the human Fc-epsilon-R1 IgE receptor protein of natural sequence, e.g. more than about twice the affinity observed for the receptor protein of natural sequence, e.g. about 7 times the affinity, as in the case for example of the asp159lys (D159K) mutant.

Since the natural affinity between IgE and the alpha-chain of the human Fc-epsilon-R1 IgE receptor protein of natural sequence is already of a high order, it is surprising and unexpected that a substantially or usefully higher affinity can be achieved by mutation, as is disclosed here.

The effect of a given mutation on the IgE affinity of a polypeptide can be tested in any of a number of convenient ways, for example using kinetic techniques as described herein below, and/or by using the test of inhibition of

IgE-mediated mast cell degranulation, as described in McDonnell et al (1996) (cited above) (p.423), which can readily be adapted to measure the 50% inhibitory concentration of a chosen mutant polypeptide under test and/or to compare it with the 50% inhibitory concentration of a suitable control or reference material, e.g. of a soluble fragment of the alpha-chain of the human Fc-epsilon-R1 IgE receptor protein of natural sequence.

Polypeptides having sequences of substantially the sequence and conformation of at least one and preferably both of the two C2-type immunoglobulin-like domains of the alpha-chain of the Fc-epsilon-R1 IgE receptor protein are preferably based on the natural sequence carrying one or a plurality of aminoacid sequence mutations by substitution, deletion or addition. Preferably at least one of the mutations is such as to enhance the IgE affinity. Further mutations can for example be approximately neutral in their effect, e.g. mutations in regions away from the binding site identified herein, and can be mutations of e.g. conservative character, and can be made e.g. for convenience in synthesis or handling of the mutant polypeptide. (For examples of 'conservative' mutations see e.g. WO 97/14808, pp12-13, or WO 96/08512, p11). Resulting polypeptides can readily be tested for IgE affinity using the test techniques described or referred to herein.

The description given by way of example below shows certain examples of mutations in the soluble fragment of the alpha-chain of the mast-cell Fc-epsilon-R1 IgE receptor protein, that substantially decrease the binding affinity of the polypeptide for IgE. While the use of such polypeptides carrying such mutations is believed to be in many cases less preferred or contra-indicated for the purposes of binding IgE and inhibiting mast cell activities as described herein, nevertheless such weaker-binding polypeptides themselves can be useful for example as comparative reagents in the performance of affinity tests and in the raising of comparison antibodies against the receptor protein, and they are accordingly representative of an aspect of the invention disclosed herein.

Also provided by the present invention is the use of polypeptides as referred to above as anti-allergic agents and in and for the manufacture of pharmaceutical compositions for use as medicaments in the preventive or other treatment or management of allergy. They can particularly for example

be used prior to an expected exposure to an agent in respect of which the subject of treatment expects to suffer an atopic (allergic) reaction. In this use, e.g. to mitigate such an atopic reaction, the polypeptides can present an advantage in not stimulating or not appreciably stimulating any antibody response, e.g. response against an idiotype determinant.

Also provided by the invention is the use of mutant polypeptides related to soluble IgE-receptor alpha-subunits and with higher affinity for IgE, to neutralise the circulating IgE attachment to endogenous IgE receptors e.g. receptors for IgE located on mast cells.

Another effect of the mutant receptor analogues is to downregulate IgE synthesis by binding to IgE expressed on the surface of B cells committed to IgE production, in a manner similar to the effect of the mutated recombinant fragments as reported by Yanagihara et al (1994) (above).

Also provided by the invention are assays such as diagnostic assays in which a receptor analogue polypeptide according to the invention is used to bind IgE as a specific binding partner for the IgE Fc region. Thus the polypeptides can also be used as binding reagents for the detection and/or determination of IgE of the corresponding species in specific binding assays. The invention therefore extends to specific binding reagents including coupling products of the polypeptides with for example labelling reagents, radioactive, enzymatic, or other per-se known labelling reagents, and/or solid-phase carriers of per-se known kind.

Also provided by the invention among the materials and methods for the production of the polypeptides discussed herein are corresponding nucleic acids encoding them, particularly nucleic acids in which the mutations discussed herein have been carried out on the basis of the known nucleic acids obtained from human and non-human animal sources encoding natural unmodified FceRI / FcϵRI receptors; and constructs in which such nucleic acids are included, such as plasmids, transfected host cells, of human or other mammalian as well as non-mammalian type, and vectors containing such nucleic acids, e.g. viral vectors such as adenoviral or herpesviral vectors.

Detailed description

Further details for performance of the invention, including description of

preparation of mutants of sFcεRIα soluble protein, are given below for illustration and not for limitation, with reference also to the accompanying drawings in which

Figure 1 shows sensorgrams, obtained as described below, for the kinetics of IgE interacting with wild-type and mutant sFcεRIα, and in particular shows substantially increased affinity of mutant D159K for IgE.

The following non-limitative description illustrates polypeptides as mentioned above; materials and methods used for their production, for example DNA and expression vectors encoding them; their expression by recombinant DNA technique. Their derivatives and pharmaceutical compositions containing them can be formulated for example according to standard technique for the formulation of sterile injectable biologicals.

Uses of such materials, for example in the modulation of atopic and other IgE-related immunological responses can be carried out by administering such pharmaceutical formulations to subjects to be treated in doses that either locally or systemically approach the levels of IgE found in vivo as can be measured for example by immunotesting blood samples with heterologous anti-IgE(Fc) antibody. Representative human dosing levels for example can be of the order of 0.01 mg/kg in respect of the soluble fragment of the Fc-epsilon-R1 alpha-chain.

A preferred example of the invention given below relates to the asp159lys (D159K) mutation of sFcεRIα and materials and methods related thereto. The trp87asp (W87D) mutation and lys117asp (K117D) mutations are given for comparison and the lys128asp mutation is shown to be of substantially neutral effect on affinity when it stands alone.

Materials and Methods

(a) Cloning the human FcεRIα DNA and construction of the soluble fragment. The cDNA encoding the human FcεRIα subunit was obtained by RT-PCR from KU812 cells. Briefly, total RNA was extracted from the cells using the method of Chomczynski and Sacchi (1987). PolyA+ mRNA was prepared by oligo(dT) affinity chromatography and first strand cDNA synthesis was carried out by reverse transcriptase. The FcεRIα sequence was then amplified

by PCR using the two primers: (Forward) 5'- GCG CGC AAG CTT CAC AGT AAG CAC CAG GAG TCC -3' and (Reverse) 5'- GCG CGC GAA TTC ATC AGT TGT TTT TGG GGT TTG GC -3'. This full-length PCR product was then subcloned as a HinDIIIEcoIR fragment into the psp73 vector (Promega) and sequenced using the chain termination method of Sanger et al (1977). The truncated cDNA encoding the two extracellular domains (val1 to lys176, numbering according to Blank et al, 1989) was obtained from the full length cDNA by PCR using the two primers: (Forward) 5'- GCG CGC AAG CTT CGC CGC CAC CAT GGC TCC TGC CAT GG -3' and (Reverse) 5'- GCG CGC GAA TTC ATC ACT TCT CAC GCG GAG CT -3'. This product was then cloned as a HinDIII/ EcoRI fragment into the pEEI2 expression vector (Bebbington et al, 1992) to give the pEEI2/sFcϵRIα construct.

(b) Site directed mutagenesis of sFcϵRIα cDNA. Circular pEE12/ sFcϵRIα was used as the template for PCR of the FcϵRIα cDNA. This was done using the splice overlap extension method based on that of Ho et al, (1989). This method requires the use of two primers per mutation, one in the sense direction (S) and the other in the anti-sense direction (A). The sequences of the primers used were, with the mutated sequences underlined:

(S) W87D: 5'- TTC AGT GAC GAC CTG CTC CTT -3':
(A) W87D: 5'- AAG GAG CAG GTC GTC ACT GAA -3';
(S) K117D: 5'- GAT GTG TAC GAC GTG ATC TAT -3';
(A) K117D: 5'- ATA GAT CAC GTC GTA CAC ATC -3';
(S) K128D: 5'- GAA GCT CTC GAC TAC TGG TAT -3';
(A) K128D: 5'- ATA CCA GTA GTC GAG AGC TTC -3';
(S) D159K: 5'- TGG CAG CTG AAG TAT GAG TCT -3';
(A) D159K: 5'- AGA CTC ATA CTT CAG CTG CCA -3'.

The sequences of the primers used for PCR amplification of the mutated products were:

5' primer: 5'- GCT GAC AGA CTA ACA GAC TGT TCC -3';
3' primer: 5'- CAA ATG TGG TAT GGC TGA -3'.

PCRs were carried out using Promega Taq polymerase in Ix reaction buffer (50 mM KCl, 10 mM tris-HC1, pH 9 at 25°C, and 0.1% (v/v) Triton X-100), with primers at a concentration of 0.2 mM, and in the presence of 0.5% Tween 20 (v/v) and 1.5 mM MgC12. Reactions contained 0.25 units of Taq polymerase per 20 μl of reaction and 1-5 ng of template per reaction. Routinely used cycle conditions were 94°C for 40 seconds, 55°C for 40 seconds and 74°C for 90 seconds. These conditions were generally used for 15-25 cycles.

HinDIII and EcoRI sites in the mutated products facilitated ligation into pEE12. DNA sequences of the sub-cloned products were checked using chain termination DNA sequencing (Sequenase, USB, Amersham, UK).

(c) Transfection and expression of wild-type and mutant sFcεRIα in NSO cells. Prior to selection of transfected colonies. mouse myeloma NSO cells (ECACC., Porton Down, UK.) were grown in CB2-DMEM (Gibco BRL) with 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin G sodium and 100 ug/ml streptomycin sulphate. Cells were incubated at 37°C with 5% CO2, and were removed from the tissue culture flasks by sharply tapping the flask sides. After colony selection, NSO cells were grown in a glutamine free medium, CB2-DMEM with 10% dialysed FBS, 100 units/ml penicillin G sodium and 100 mg/ml streptomycin sulphate.

Stable transfections of NSO cells were carried out by electroporation using a Bio-Rad gene pulser electroporator. For each transfection, 1 x 10^7 exponentially growing cells were pelleted at 1500 rpm for 5 mins. These were then washed twice in ice cold I x PBS, and resuspended in 0.7 ml of ice cold Ix PBS. 40 μg of linearised pEE12 DNA was then added, typically in a 100 μl volume of a Sall restriction enzyme buffer. The DNA/cell suspension was transferred to a pre-chilled 0.4 'cm electrode gap electroporation cuvette (Bio-Rad), and left on ice for 5 mins. The sample was electroporated by two consecutive pulses of 1500 V at 3 μF. The cuvette was then returned to ice for 5 mins before the cell suspension was diluted in 140 mls of CB2-DMEM medium including 2 mM L-glutamine as described above. The cell suspension was then plated into 14 96 well plates at 100 μl per well and left overnight at 37°C. The following day methionine sulphoxamine was added in a volume of

100 μl per well to give a final concentration of 5 mM. Plates were left for 24 weeks to allow selection to take place and selected colonies were then expanded for roller culture.

Cells were seeded at a concentration between 1 x 10^5 and 1 x 10^6 in a maximum volume of 250 mls. Cells were split or diluted upon reaching a cell density of 1 x 10^6 ml. Cells were gassed for two minutes each day with a gas mixture of 5% CO2, 20% O2 and 75% N2. When cell numbers had remained constant for 3 days, the supernatants were harvested by centrifugation at 10 000 g (Sorvall RC-2B plus centrifuge, GSA rotor) at 4°C. Culture supernatants were then passed through a 0.45μm filter (Millipore) and stored at 4°C in the presence of 0.1% sodium azide (Sigma).

(d) Purification of wild-type and mutant sFcεRIα using the 3B4 anti-FcεRIα mAb. The 3B4 hybridoma produces an anti-FcεRIα α (1) domain monoclonal antibody of the IgG1 subclass (M. Hogarth, personal communication). The cell-line was initially grown in DMEM (Gibco BRL) with 10% FBS, 2 mM L-glutamine. 100 units/ml penicillin G sodium and 100 mg/ml streptomycin sulphate. The line was then adapted for growth in serum free conditions by a progressive reduction in the amount of FBS, and a corresponding increase in the amount of Hybridoma-SFM medium (Gibco BRL) in the growing cultures. The Hybridoma-SFM was supplemented with 2 mM L-glutamine and 100 units/ml penicillin G sodium and 100 mg/ml streptomycin sulphate. 3B4 was purified using Protein A sepharose (Sigma), and an anti-FcεRIα affinity column was made from the purified material by covalently immobilising it to CNBr-activated sepharose (Pharmacia) according to the manufacturer's instructions.

Harvested sFcεRIα supernatants were circulated over the affinity column overnight at approximately 60 ml/hour, and the column was then washed with at least 10 bed volumes of PBS. Bound protein was eluted with 0.2 M glycine pH 2.5. The eluted protein was neutralised with I M Tris and stored ready for further use. Proteins were analysed by 12% SDS PAGE Laemmli, 1970) stained with ISS Pro-Blue (Enprotech. Hyde Park, MA, USA) and showed expected molecular weights on SDS PAGE compared with Rainbow (TM) standards (Amersham, UK).

(e) CD analysis of wild-type and mutant sFcεRIα: CD studies were

performed on a Jobin-Yvon CD6 spectrophotometer (Longjumean, France). Wild-type and mutant s sFcϵRIα samples were analysed in cylindrical quartz cells of 0.5 mm path length. The spectrophotometer was calibrated for wavelength and ellipticity using d-10-camphorsulphonic acid. Measurements could be taken at a sample concentration in the range of 100-500 μg/ml in 20 mM sodium phosphate buffer, pH 7.4, at constant temperature in a thermostatted cell holder. All samples here were measured at 400 μg/ml in the stated buffer, in a 0.5 mm path length cell at 4°C. Blanks consisted of buffer-only samples and their spectra were subtracted from wild-type and mutant sFcϵRIα sample measurements. Spectra were averaged over five repeated scans, each carried out in 0.2 nm steps, with an integration time of 1 second. The mean residue weight ellipticity, $\Delta\epsilon$ (= ϵL - ϵR dm3 mol-1 cm-1), was calculated from the molar residue ellipticity, [θ], by the following relationship:

$$\Delta\epsilon = \frac{[\theta]}{3298} \qquad \text{(equation 1)}$$

The molar residue ellipticity was determined from the observed ellipticity, e. by the following relationship:

$$[\theta] = \frac{[\theta]Mr}{100/c} \qquad \text{(equation 2)}$$

where θ is the observed ellipticity (in degrees), l is the optical path length (decimetres), c is the concentration of the sample (g/ml). and Mr is the mean residue molecular weight (g/mol), determined from the amino acid composition. The CD spectra were analysed with and presented by MicroCal Origin (Version 3. MicroCal Software. MA, USA) graph drawing software. Results from wild-type sFcϵRIα and mutants of sFcϵRIα (K117D) and (D159K) were rather closely superimposable and showed that the mutations had not appreciably disturbed the overall molecular conformation.

(f) Preparation of the SPR sensor surface: Purified sFcϵRIα was coupled

to CM5 sensor chips using the aldehyde coupling reaction according to manufacturer's instructions. Briefly, the α-chain was oxidised to introduce aldehyde groups by incubating 100-200 μg of protein with 1mM sodium metaperiodate (Sigma) on ice for 20 mins, in 100 mM sodium acetate buffer, pH5.5. The sensor chip was then activated using a solution of 50 mM N-hydroxysuccinimide (NHS) and 200 mM N-ethyl-N'-(dimethylaminopropyl) carbodimide (EDC). 5 mM carbohydrazide (Sigma) in 10 mM sodium acetate buffer, pH 4.0, was then injected over the activated surface. This step bound carbohydrazide to the active esters on the chip surface. Any residual esters were inactivated by an injection of 1 M ethanolamine hydrochloride, pH 8.5. The modified sFcϵRIα was then injected over the sensor chip until a sufficient quantity had bound. A range of 100 - 10000 RU was initially tested, and for the data shown in this paper an immobilisation density of 500 - 1000 RU was used. This low level of immobilised protein is necessary to prevent mass transport effects distorting kinetic measurements. The hydrazone bond formed during aldehyde coupling is unstable at low pH, and was thus reduced with 0.1 M sodium cyanoborohydride in 0.1 M acetate buffer, pH 4.0, to enhance its stability. This prevented any loss of the immobilised sFcϵRIα during regeneration of the sensor surface with 0.2 M glycine at pH 2.5, which was carried out between experiments to remove all non-covalently bound proteins.

(g) SPR analysis of wild-type and mutant sFcϵRIα. Three proteins were used to probe the interaction with wild-type and mutant sFcϵRIα. IgE-WT (Burt et al, 1986) is a myeloma protein, purified as described for IgE-Fc (Young et al, 1995). IgE-Fc in which the glycosylation sites at Asn265 and Asn371 have been mutated to Gln, has been described in detail elsewhere (Young et al, 1995). Fcϵ3-4 is a covalently linked dimer consisting of the last two residues of Cϵ2 and the entire Cϵ3 and Cϵ4 domains of IgE. It is secreted from NS0 cells as a dimer due to the presence of the interchain disulphide bond at Cys328 (Shi et al, 1997).

All interactions measured using the BIAcore were carried out at 25°C, using HBS (BIA certified, being 10 mM Hepes, pH 7.4, 150 mM NaCl, 3.4 mM EDTA and 0.005% v/v surfactant P-20) as the continuous flow buffer, generally at a flow rate of 10 μl/min. A wide concentration range of analytes

(IgE, IgE-Fc and Fcϵ3-4) was used for the kinetic analyses: 250 nM, 125 nM, 62.5 nM, 31.25 nM and 15.63 nM, unless otherwise indicated. The analyte was injected For 150 seconds, followed by HBS for approximately 600 seconds to monitor the dissociation of bound analyte. The chip was then regenerated with three 60 second pulses of 0.2 M glycine at pH 2.5. The glycine washes had no effect upon the subsequent activity of the chip. Non-specific binding of the ligand to the sensor surface was assessed by performing sample injections onto a sensor surface which had no protein coupled to it. Under these conditions there was negligible non-specific binding for all of the ligands tested.

The interactions of wild-type and mutant sFcϵRIα with IgE were monitored at five IgE concentrations which were 250 nM, 125 nM, 62.5 nM, 31.3 nM and 15.6 nM. A 150 second association phase was followed by a 600 second dissociation phase with HBS buffer flowing over the sensor surface at 10 μl/min. Representative sensorgrams are shown in Figure 1, illustrating high affinity in the D159K mutant.

(h) Kinetic analysis of SPR data. Data were analysed using the BIAevaluation analysis package (version 2.1, Pharmacia Biosensor). Non-specific binding was subtracted from the specific binding prior to kinetic analysis. Simulations of sensorgrams, using kinetic parameters derived from the kinetic analysis were obtained using BIAsimulation software (version 1.1, Pharmacia Biosensor). SPR data were also obtained for IgE binding to immobilised wild-type sFcϵRIα or sFcϵRIα (K117D).

Results

(a) Characterisation of wild-type and mutant sFcϵRIα. Four mutations were introduced independently into the membrane-proximal α(2) domain of FcϵRI. W87D in strand A, K117D in strand C, K128D in strand C' and D159K in strand G. Recombinant wild-type and mutant sFcϵRIα. proteins were affinity purified using the anti-FcϵRIα mAb 3B4 which recognises an epitope in the α(1) domain.

The products were then assessed for purity by SDS PAGE and found to

exhibit the typical broad bands observed by others and attributed to heterogeneous glycosylation of the α-chain (Blank et al, 1991: Keown et al, 1995; Letourner et al, 1995). Small differences in the mean electrophoretic mobility of the recombinant products may reflect glycosylation differences that result from variations in the culture conditions (Hayter et al, 1993). The purified proteins were assessed by SPR for immunoreactivity with the anti-FcϵRIα mAb 15.1, which recognises an epitope in the sFcϵRIα α(2) domain, and is inhibitory for IgE binding (Wang et al, 1992). All of the sFcϵRIα. mutants bound the same amount of mAb 15.1 as wild-type sFcϵRIα., implying the maintenance of this epitope (data not shown).

CD spectra were recorded over the range 195 to 260 nm to assess the conformation of the sFcϵRIα mutants. sFcϵRIα is expected to consist of β-sheet structure since it is predicted to comprise two immunoglobulin-like domains (Padlan and Helm, 1992; McDonnell et al, 1996). A positive signal at 203 nm and negative signal at 215 nm, observed in the spectrum of wild-type sFcϵRIα, are indicative of the predicted beta-structure, while the positive signal at 230 nm may reflect contributions from the disulphide bonds present in sFcϵRIα, and also the high density of surface aromatic residues. This spectrum is essentially identical to those published previously (McDonnell et al, 1996; Sechi et al, 1996).

The CD spectrum of sFcϵRIα (K117D) exhibited a slightly greater ellipticity at 230 nm, but the 215 nm peak and 203 nm trough, which are representative of the beta-structure, are identical to wild-type sFcϵRIα. The spectra of the W89D, K128D and D159K mutants were identical to wild-type sFcϵRIα over the entire recorded spectrum, indicating that these mutations have caused no structural perturbation whatsoever.

(b) Kinetics of binding of wild-type and mutant sFcϵRIα to IgE, IgE-Fc and Fcϵ3-4. The kinetics of binding of IgE. IgE-Fc and Fcϵ3-4 to immobilised wild-type and mutant sFcϵRIα was assessed by SPR. Figure 1 shows sensorgrams for the interaction of IgE with the wild-type and mutant receptors. It may be seen that there is a large difference between the sensorgrams for wild-type sFcϵRIα and sFcϵRIα (K117D). Not only is there a lower response (reflected in the number of RUs recorded), but the off-rate is

clearly much faster for this mutant than for wild-type sFcεRIα. It can also be seen that the dissociation of IgE from sFcεRIα (DI59K) is slower than that from wild-type sFcεRIα. These trends persist in the sensorgrams for the interactions of IgE-Fc and Fcε3-4 with the wild-type and mutant sFcεRIα (data not shown).

Initial fitting of data was carried out using monophasic models of association and dissociation. However, it was clear that the data could not be satisfactorily described by a simple monophasic model, while an excellent fit was obtained with biphasic models of association and dissociation. This was seen in a data analysis for the interaction of IgE with wild-type sFcεRIα (Figure 4 in Cook et al, 1997), and sFcεRIα (K117D). Residual values for the monophasic fits are very high and non-randomly distributed along the curve, whereas those for the biphasic fits are considerably smaller and are randomly distributed. Similarly, the binding of IgE and Fc fragments to all of the mutants were best fitted by a biphasic model.

The biphasic fits are characterised by two association rates and two dissociation rates, and these values yield an affinity constant for each phase of the interaction (Table 1 in Cook et al, 1997) These may be characterised as a fast. low affinity. and a slow, high affinity interaction. The Ka2 value for the latter interaction of 2.7 x 10^9 M-1 is slightly lower than the values obtained from cell binding assays which are of the order of 10^10 M-1 (Basu et al, 1993; Hakimi et al, 1990; Keown et al, 1997, Mallamaci et al, 1993; Young et al, 1995) The Ka2values determined for IgE-Fc and Fcε3-4 are of the same order as those for IgE, namely, 3.3x10^9 M-1 and I.5x10^9 M-1, respectively.

IgE exhibits a 30-fold lower affinity for sFcεRIα (K117D) and a 7-fold higher affinity for sFcεRIα (D159K) than for wild-type sFcεRIα (Table 1) The dramatic effect on the affinity of IgE for sFcεRIα (K117D) is due principally to a 145-fold increase in the dissociation rate of the second component of the interaction. (kd2 = 4.6x10^-3 s-1, compared with 3.2x10^-5 s-1 for wild-type sFcεRIα). Similarly. the 7-fold increase in the affinity of IgE for sFcεRIα (D159K) is due principally to a 5-fold decrease in the dissociation rate kd2 relative to wild-type sFcεRIα (6.3x10^-6 s-1 and 3.2x10^-5 s-1, respectively). The kinetic parameters of the two remaining mutants, sFcεRIα (W87D) and sFcεRIα (K128D), are also described in Table 1. sFcεRIα (K I28D) is

indistinguishable from wild-type sFcεRIα, but sFcεRIα (W87D) displays a modest decrease in the affinitv of the second component (Ka2), due to a 12-fold increase in the dissociation rate kd2 (3.9x 10^-4 s-1 compared with 3.2x10^-5 s-1 for wild-type).

The K117D mutation also has a dramatic effect on the ratio of the two components which can be estimated from the kinetic analysis. The parameter R0 represents the total binding signal at equilibrium, while R1 is the binding due to the fast, low affinity phase; thus the ratio R1/R0 (Table 1) is the fractional contribution of the fast; low affinity, phase. These values indicate that for the interaction between wild-type sFcεRIα and wild-type IgE, the slow, high affinity phase dominates overwhelmingly at 94% of total binding. However, the K117D mutation alters the ratio such that only 26% of total binding is contributed by the slower, high affinity phase, and for this mutant, the faster, low affinity phase predominates. This reversal of the relative contribution of the fast and slow phases to the overall binding was seen in simulated curves (Figure 5 in Cook et al 1997) based upon experimentally derived kinetic parameters. These curves are discussed further below.

(c) Kinetics of binding of wild-type and mutant sFcεRIα to IgE-Fc(R334S). IgE-Fc(R334S) is a mutant of IgE-Fc in which Arg334, at the N-terminal end of strand A in Cε3, has been substituted by serine. This mutation greatly increases the rate of dissociation from the complex with membrane-bound or immobilised sFcεRIα, resulting in a 120-fold decrease in the affinity for the receptor (Henry et al, 1997, accompanying paper). Kinetics of binding of IgE-Fc(R334S) to wild-type sFcεRIα and the four mutants were examined (Figure 6 of Cook et al, 1997). The extent of binding of IgE-Fc(R334S) to wild-type sFcεRIα is significantly diminished relative to that of wild-type IgE-Fc, (Ka2= 2.9x10^7 M-1 compared with 3.3x10^9 M-1 for wild-type IgE-Fc) as previously observed. The extent of IgE-Fc(R334S) binding to sFcεRIα (WS7D) and sFcεRIα (K117D) is barely detectable (Figure 6 of Cook et al, 1997), and too low for any kinetic parameters to be determined. These further reductions in binding, in excess of that seen with wild-type sFcεRIα, indicate that these three residues (Arg334 in IgE-Fc, Trp87 in sFcεRIα and Lys117 in sFcεRIα), all of which are obviously contact residues, do not interact with each other, and must contribute independently to the interaction.

The KI28D mutation has no effect upon the binding of wild-type IgE-Fc (Ka2 = 4.5x10^9 M-1, Table1), but the affinity of IgE-Fc(R334S) is significantly diminished (Ka2 = 6.5x10^6 M-1 Table 2). The D159K mutation, however, which enhances the binding of wild-type IgE-Fc (Ka2 = 1.9x10^10 M-1 compared with 3.3x10^9 M-1 for wild-type sFcϵRIα. Table 1), appears also to enhance the binding of IgE-Fc(R334S) to a similar extent (Ka2 = I.3x10^8 M-1 compared with 2.9x 10^7 M-1 for wild-type sFcϵRIα, Table 2).

In particular, the examples show that the effect of substituting Asp 159 by lysine causes an increase in the affinity for IgE, by a factor of seven. Not only does this confirm Asp159 as close to, or part of the site, but it shows that the high affinity of the IgE-FcϵRI interaction can be further enhanced.

The results presented here identify Lys117 as a contact residue, and both Lys128 and Asp159 as being near to the IgE binding site in sFcϵRI. Two of these mutations (Lys117 and Asp159) are shown in the predicted structure of (s)FcϵRIα (McDonnell et al., 1996). The peptide Ile119-Tyr129 has been shown to inhibit IgE binding to sFcϵRIα (McDonnell et al., 1996). Residue Lys128 lies within this sequence. Superimposed on this structure are the results of Danho et al. (1995) and Mallamaci et al. (1993). The former authors identified residues in a(2) to be important for IgE-binding (Val115, Lys117, Val118, Tyr120, Tyr121, Lys122, Asp123, Tyr129, Tyr131, Tyr149, Gly153 and Val155). Mallamaci et al. (1993) focused on the a(1) domain and identified regions that are not involved in binding IgE. All of the regions, highlighted by Mallamaci et al., are distal from a(2) in the predicted structure of the a-chain and this also would point to the loops proximal to the a(2) domain as involved in IgE-binding. It can be seen that the two binding regions are of similar extent.

The invention extends to pharmaceutical compositions comprising the modified FceRIa or sFcϵRIα polypeptides provided hereby; and to methods of medical treatment, utilising the modified FceRIa or sFcϵRIα polypeptides provided hereby. For example a modified polypeptide according to the invention can be given parenterally, e.g. at a level within an order of magnitude of the 0.01mg/kg level indicated hereinabove, e.g. systemically or

by direct injection into a tissue that is the subject of excessive IgE action such as an allergic reaction, for the reduction of IgE effects, such as allergic reactions, in vivo.

The invention also extends to methods and diagnostic kits for testing materials that are binding partners for IgE-Fc or FceRI / sFcϵRIα, utilising the modified FceRIa polypeptides provided hereby, e.g. a method for binding a IgE polypeptide, comprising exposing a preparation containing the IgE polypeptide to a modified FceRIa or sFcϵRIα polypeptide as provided hereby: e.g. for testing or isolation; and a method for purifying the modified FceRIa / sFcϵRIα polypeptides provided hereby, by binding them with IgE or its Fc or constant-domain derivative, e.g. in an immobilised form such as a derivatised polymer column material. The complex formed in the process can then be dissociated by high salt concentration such as is used for dissociating protein complexes and the desired protein obtained freed from its binding complex.

The invention also extends to fusion proteins comprising the extracellular domains of altered FceRIa / sFcϵRIα polypeptides as described herein, fused in per-se known manner e.g. at their C-terminal to a desired peptide or polypeptide, and other mutations retaining sFcϵRIα function.

Examples of modified polypeptides according to the invention involve other than mutations of trp130, trp156, tyr160, or glu161 in FceRIa or sFcϵRIα: especially other than the products of substituting those residues by alanine residues.

Examples of modified polypeptides according to the invention also involve other than mutations of tyr131, glu132, val155, leu158, or asp159: especially other than the products of substituting those residues by alanine residues.

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The invention disclosed herein is susceptible of modifications and variations as will be readily apparent to persons skilled in the art. The present invention and disclosure extends to modifications and variations and to combinations and subcombinations of the features mentioned or disclosed in the present description and/or claims or shown or indicated in or by the accompanying Figures. Documents cited herein are hereby incorporated by reference in their entirety for all purposes.

Claims:

1: A soluble polypeptide having an IgE-binding functionality corresponding to that of the alpha-chain of the mast-cell Fc-epsilon-R1 IgE receptor protein, and of enhanced binding to the Fc portion of said IgE, said polypeptide (a) comprising a sequence of substantially the sequence and conformation of at least one and preferably both of the two C2-type immunoglobulin-like domains of the alpha-chain of the Fc-epsilon-R1 IgE receptor protein, and (b) substantially lacking a sequence corresponding to the transmembrane segment of said receptor protein, and (c) also having a mutation, relative to a natural sequence of said receptor protein, in one or more of the aminoacid residues of the C'CFG region of the FceRI subunit: (in the C strand): asp114, val115, lys117, ile119, tyr121, lys122, and asp123: (in the C' strand): gly124, glu125, ala126, leu127, lys128, tyr129, tyr131, and glu132: (in the F and G strands, including the F-G loop): glu144, asp145, gly147, thr148, tyr150, thr152, lys154, gln157, leu158, asp159, ser162, glu163, pro164, leu165, asn166, thr168 and ile170.

2: A polypeptide according to claim 1, having a mutation in one or more of the aminoacid residues lys117, ile119, tyr121, gly124, glu125, ala126, leu127, lys128, tyr129, tyr131, glu132, lys154, trp156, gln157, leu158, and asp159.

3: A polypeptide according to claim 2, having a mutation of one or more of the following kinds: (a) a mutation of lys117 to a large hydrophobic residue such as phe, tyr or trp; (b) a mutation of glu132 to a large hydrophobic residue such as tyr; (c) a mutation of lys154 to a large hydrophobic residue such as phe, leu, or tyr; (d) a mutation of gly124 to a positively charged residue such as lysine or arginine; (e) a mutation of lys128 to a negatively charged residue such as glutamate; (f) a mutation of gln157 to a negatively charged residue such as glutamate or aspartate; (g) a mutation of leu158 to a negatively charged residue such as glutamate or aspartate; (h) a mutation of asp159 to a positively charged residue such as lysine or arginine.

4: A polypeptide according to claim 1, having a mutation of one or more of the following kinds: mutation of any of the following hydrophobic residues to a hydrophobic residue of similar or larger size: ile119 to leu or phe; tyr121 to phe or trp; ala126 to val, leu or ile; leu127 to ile or tyr; or tyr129 to phe or trp; or mutation of any of the following residues to form or enhance a salt bridge interaction: glu125 to asp; tyr131 to asp or glu; or asp159 to lys or arg.

5: A nucleic acid encoding a polypeptide according to any one of claims 1-4, for example (a) a nucleic acid in which the mutation has been carried out on the basis of a known nucleic acid obtained from a human source encoding natural an unmodified FceRI receptor; or (b) a construct in which such a nucleic acids are included, such as a plasmid, a transfected host cell of human or other mammalian as well as non-mammalian type, or a vector containing such a nucleic acid, e.g. a viral vector such as an adenoviral or herpesviral vector.

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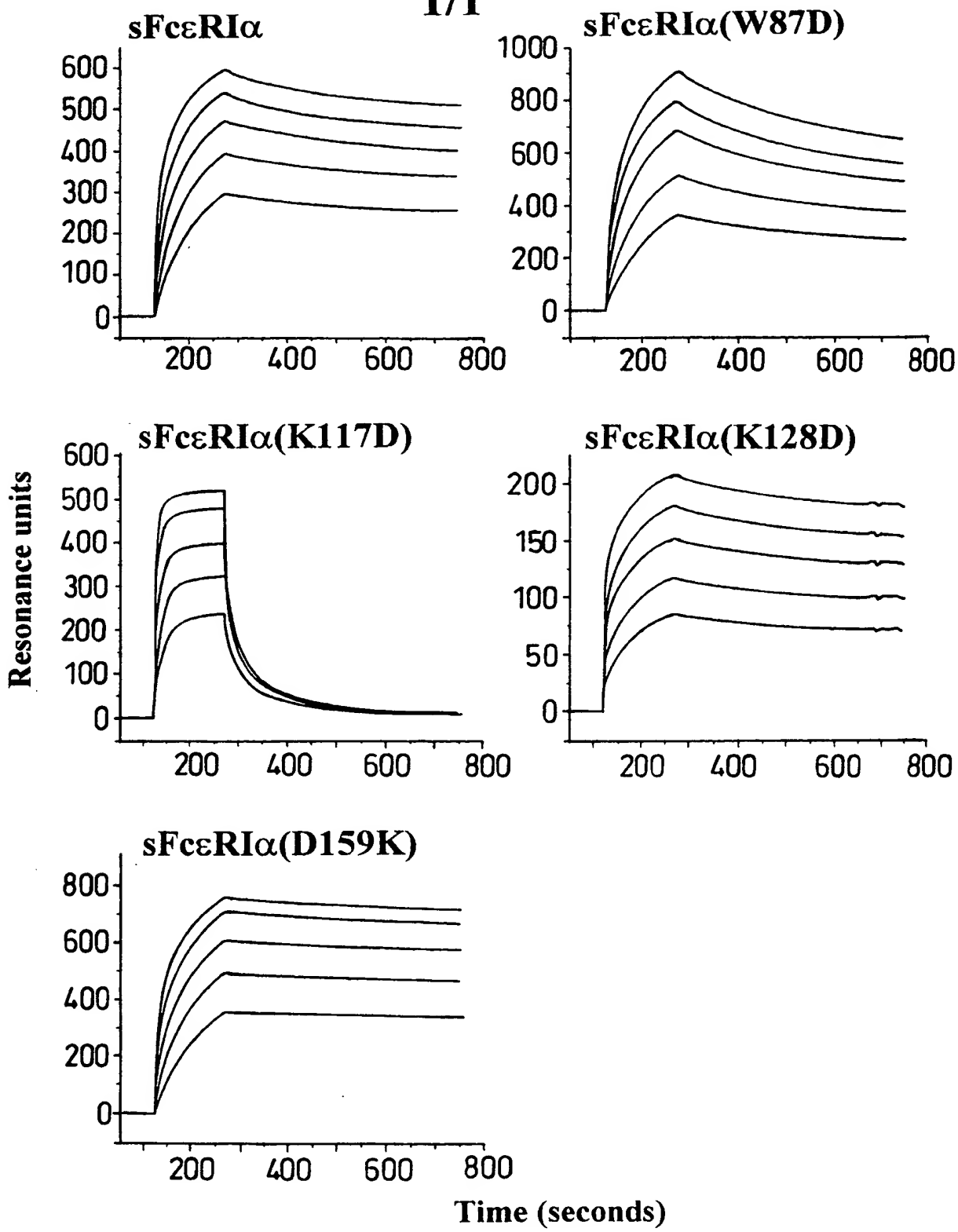

Fig. 1

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 98/02207

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MCDONNEL, J.M. ET AL.: "Structure based design of peptides that inhibit IgE binding to its high affinity receptor Fc epsilon RI." IMMUNOLOGY, vol. 89, no. Suppl.1, 1996, pages 2-Abstr.SC10, XP002086800 see abstract	1,2,5
O,X	& MCDONNEL, J.M. ET AL.: "Structure based design of peptides that inhibit IgE binding to its high affinity receptor Fc epsilon RI." JOINT CONGRESS OF THE BRITISH SOCIETY FOR IMMUNOLOGY AND THE BIOCHEMICAL SOCIETY, 10 - 13 December 1995, Harrogate, England, UK see the whole document	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 December 1998	28/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	SMALT, R

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MALLAMAC, M.A: ET AL.: "Identification of sites on the human Fc-epsilon-RI-alpha subunit which are involved in building human and rat IgE" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 29, 15 October 1993, pages 22076-83, XP002086801 see figures 1,3 ---	1-5
X	WO 96 08512 A (AUSTIN RESEARCH INST CANCER AN ;HOGARTH PHILLIP MARK (AU); MCKENZI) 21 March 1996 cited in the application see page 12, line 25 - line 32 see page 8, line 26 - line 33 see page 13, line 11 - line 12; claims 7,8,19,32; examples 4,5 ---	1,2,5
X	DANHO, W. ET AL.: "High affinity IgE-Receptor-alpha-subunit derived peptides as antagonists of human IgE binding." 9TH INTERNATIONAL CONGRESS OF IMMUNOLOGY, 23 - 29 July 1995, pages 677-Abstr.4018, XP002086802 San Francisco, California cited in the application see the whole document ---	1,2,5
A	WO 96 01643 A (UNIV JEFFERSON ;KING S COLLEGE (GB); JAMESON BRADFORD A (US); SUTT) 25 January 1996 cited in the application see the whole document ---	
P,X	COOK, P.D. ET AL.: "Identification of contact residues in the IgE binding site of human Fc-epsilon-RI-alpha." BIOCHEMISTRY, vol. 36, 16 December 1997, pages 15579-88, XP002086803 see the whole document -----	1-5

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/GB 98/02207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 3557295 A	29-03-1996
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